Allosteric modulation on *In-silico* Molecular Docking Studies Vitamin D receptor specific PUFAs in Type 2 Diabetes

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Abstract:

Background: Type 2 Diabetes mellitus (T2DM) serious health threat affecting the group as essentially every country around the globe, with the prevalence of Type-2 Diabetes.T2DM taking place over a long period of time to the peripheral insulin resistance appropriate to the inactivation of the Insulin Receptor protein. Elucidated compounds should be evaluated in favor of the use of an antidiabetic in-silico docking study. Active site and pocket-finder apparatus shall be analyzed for the receptor. Amino acids were predicted to be active site binding residues. Docking studies were carried out during the Schrödinger software system. **Aim:** To the present study identified omega-3 PUFAs (EPA DHA & AA) Compounds while important blocker of hydrophobic pocket throughout molecular modeling study beside Type2 Diabetes. **Materials and Methods:** A group of three analog VDRs are being developed for discovery treatment with a type 2 diabetes inhibitor. Its use as a molecular docking study was to recognize the binding method involving the compound in T2diabetes

through ADMET prediction. Whether to predict binding energy in the MMGBSA test. The molecular dynamic stimulation was enhanced by conformation within the strength of the possible composite binding. The PUFAs (EPA, DHA & AA) derived contain experience to Type 2 diabetes **Results and Discussion:** Based on the computational results, the Omega-3 and Omega-6 PUFAs EPA, DHA & AA compounds encourage energy interaction. Composite contains an in-vitro antidiabetic assay; the compounds must be clearly shown that it is active on type 2 diabetes. Conclusion: Our studies provide vital information on the findings of biomolecules of type 2 diabetes inhibitors.

Key words: Type 2 Diabetes Mellitus (T2DM), Polyunsaturated fatty acids, Vitamin D Receptor, EPA, DHA, AA.

1. Introduction:

The amount of individuals with Type 2 diabetes mellitus (T2DM) risen significantly in 2000. T2DM occurrence is estimated to be 2.8 percent. In 2030, 4.4% of the inhabitants might have been affected by diabetes. As more than just a consequence, 366 million T2 diabetics anguishes in the globe are being described throughout the last fifteen years.(Wild, Roglic, Green, Sicree, & King, et al., 2004). This virulent disease is largely due both to age of the population, steadily increasing obesity and inactive lifestyle (Van Dieren, Beulens, Van der Schouw, Grobbee, & Neal, 2010; Wild et al., 2004). T2diabetes mellitus is associated with insulin resistance during skeletal muscle and adipocyte tissue, with additionally, dysregulation of hepatic gluconeogenesis impeded insulin secretion, which was necessary for reestablishing insulin sensitivity. (Gannon, Conn, & Vaughan et al., 2015). Asian T2DM could increase Insulin sensitivity in response to n-3 polyunsaturated fatty acids supplementation although encouraging the evaluation with patients to ethnicity(D. Li,

2015).4Previously, there will be an increasing indication that perhaps the acute phase inflammatory reaction produced by cytokines is directly associated with the identification of insulin resistance and T2DM. Even though important genetic factors, it is necessary to consider the description that diabetes is a multi-factorial disorder.(J. C. Pickup and M. A. Crook et al.,1998). VD3 is a few compounds of both the metabolite 1ш, 25-dihydroxyvitamin D3, as well as the transcription factor VDR, which provide a direct influence on the control of gene expression. As with other nuclear endocrine receptors, such as significant conformation changes that have taken place from outside Ligand binding domain, were influences the The receptor's protein-protein interaction profile through nuclear adaptor proteins, while the corepressors co-activate the intermediate compound. Vitamin D receptor ligand-dependent transcriptional regulator adheres to a super family with nuclear receptors (NRSF) (Mangelsdorf et al., 1995). In the specific instance of PUFAs in Vitamin D Receptor of Type 2 DM, metabolic disorder with distinct etiology is described by typical Lipid, carbohydrate insulin action, protein metabolism caused by a deficiency in insulin secretion otherwise associated. Mainly difficult heterogeneous disease with type 2 diabetes mellitus. T2DM, typically appear in middle age, seems to have an effect of insulin insufficiency and insulin resistance. Individuals T2D contain a difficult threat. T2DM is a common disease which was also measured at around 250 x 106 overseas, in addition to being predictable to increase 380 x 106 through 2025. In recent decades, Type 2 diabetes mellitus is significantly increasing in prevalence.

2. Selected PUFAs:

2.1. Eicosapentaenoic acid is (C20H30O2), Omega 3 PUFAs have 5 cis-double bond positions (5, 8, 11, 14 and 17).EPAs have been reported to get numerous metabolic diseases and are consequently considered necessary micronutrients by the majority of individuals.(Bays HE et al., 2011).

2.2. Docosahexaenoic acid (C22H32O2) Omega 3 PUFAs containing 6 cis-double bonds at positions (4, 7, 10, 13, 16 and 19). It is by far the most critical structural component. for the individual organs. Amino phospholipids Docosahexaenoic Acid will be initiated at a high

level in a variety of sub cellular brain fractions, including nerve terminals, microsomes, synaptic vesicles, and synaptosomal plasma membranes.(Calder PC et al.,2010).

2.3. Arachidonic acid ($C_{20}H_{32}O_2$) 6 Omega long-chain PUFAs have 4 (Z)-double bonds on positions (5, 8, 11 and 14).Synthesizing as dietary linoleic acid(LA) Arachidonic acid is a synthesis substrate for a variety of physiologically active chemicals (eicosanoids) found in cell membrane phospholipids, including prostaglandins, thromboxane's, and leukotriene's. (Derek R. Buckle and colleagues, 1973). Along with the proper functioning of several organs and systems, Arachidonic acid (AA) regulates inflammation either directly or even after its alteration into eicosanoids.

3. Selected Vitamin D receptors

VDR It consists of many domains: the ligand binding domain (LBD), the DNA binding domain, the N-terminus, and the HINGE region. This could be used for dimerization. Among the numerous domains, LBD is critical for the binding of 12 helical receptors and the development of a compact three-dimensional structure, particularly when bound to a ligand. The receptor completely includes the ligand binding pocket, allowing for unique interactions with natural ligands, especially important 1, 25-dihydroxy vitamin D3. Thousands of VDR ligands contain synthesize in the current decades to combine this exact ligand binding pocket. In response to ligand, the VDR recruits its chosen dimerization associate, the RXR binding to the VD receptor elements (VDREs) Collected two hexameric binding sites, both of which are directly interspersed by a changeable number of nucleotides, although commonly 3 were often inverted palindromes interspersed through the 9 nucleotides (Haussler MR et al.,1998, Schrader M et al.,1997). Gene Seq (A/G)G(G/T)TCA should be considered in favor of the half-site Vitamin D Receptor (VDRE) element, even though there is a significant sequence multiplicity. The 2 receptors interact symmetrically through their Ligand Binding Domain together in the helix 12 activate configuration, but their DNA Binding Domain (DBD) is asymmetrical, with the Retinoic x Receptor (RXR) because of additional compactness in addition to the Vitamin D receptors (VDR)During the wide - ranging configuration of both the LBD and the DNA BD. The flexibility of the hinge region becomes almost certainly appropriate and requires the correct positioning of the receptors together for the same helix structure of the DNA. Several VD control genes include a pair of Vitamin D receptor elements (VDREs) within the promoter, irregularly

constant distant though from the coding district, as well as the chromatin immuno-precipitation study, the receptor complex binds to different Vitamin D receptor elements were exposed during repeated influencing factors. (Meyer M B, et al, 2006; Kim. S et al, 2005).

4. Research Methodology:

4.1. Materials and Methods:

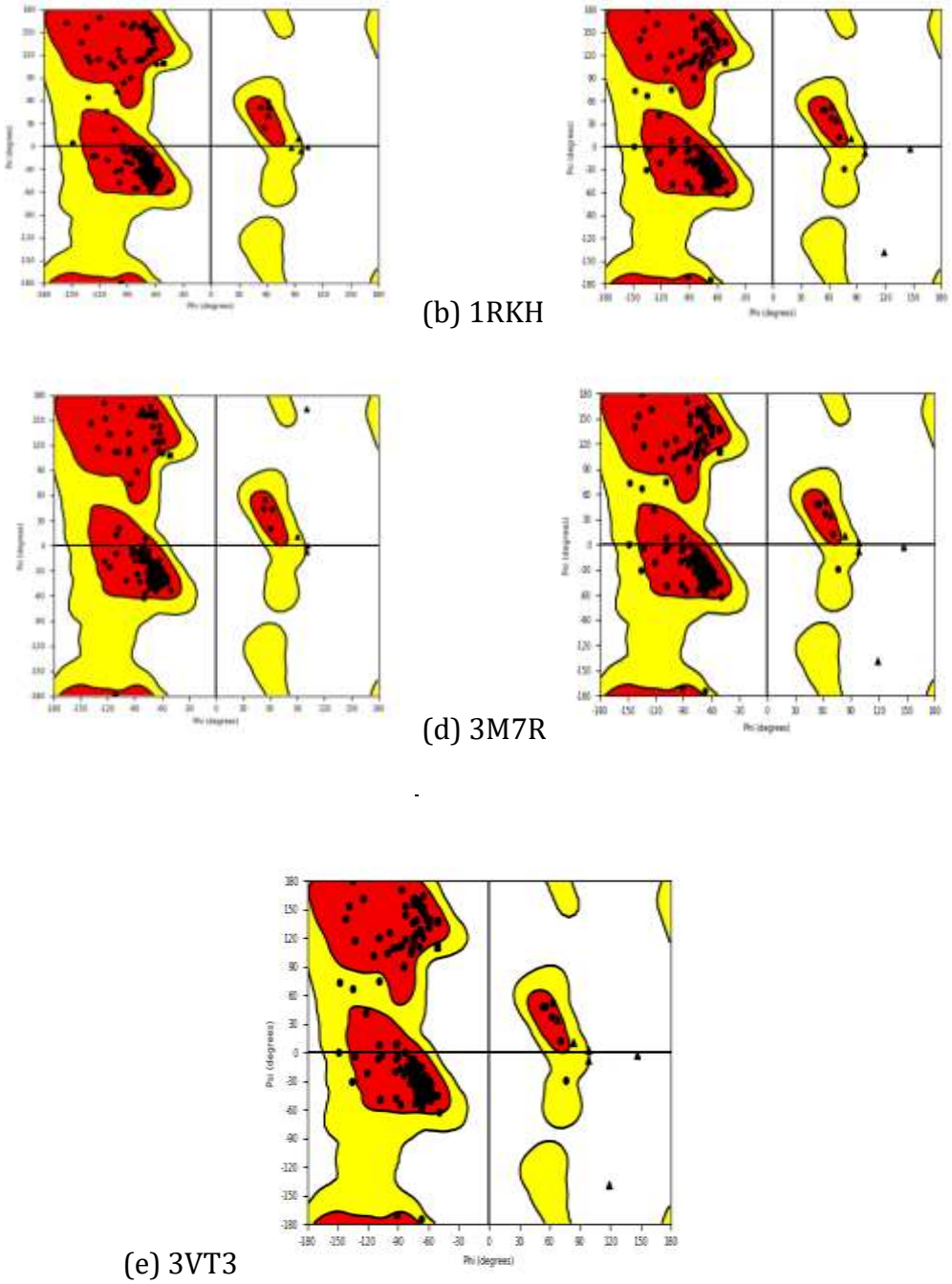
4.2. Devices and materials

To study led in-silico molecular through the use of bioinformatics tools. Numerous offline programming on the web will be used for exploration. Programming used for the examination is ACD/Labs' ChemSketch 12.01, study Collaboratory used for Structural Bioinformatics (RCSB) Protein Data Bank (PDB).The materials used in this evaluation were information on the arrangement and 3D structure of selected VDRs. The drug relevance was checked using the molinspitation toolkit(http://www.molinspiration.com/)This information are accessible online under National Center of Biotechnology Information (NCBI) (http://www. ncbi.nlm.nih.gov),European Molecular Biology Laboratory-EBI (http://www.ebi.ac.uk/) and PDB at the RCSB site(http://www. rcsb.org/pdb/home/home.do),For a molecular docking analysis of the three fatty acid compounds against by Vitamin D Receptors, the Schrodinger Software System docker and glide module were accomplished.

4.3. Preparation of protein:

The information of three-dimensional structural macromolecules was retrieved from the website (http://www. rcsb.org/pdb). From the PDB, Vitamin D receptor (VDR) Including the Protein Data Bank RCSB Id: 1RKG, 1RKH, 3M7R, 3VT3, 3VT7),(Berman et al., 2014). with a resolution of ($2.1A^0$) were obtained. The crystallographic water molecules were reduced as protein followed by the addition of missing hydrogen atoms. Alternate confirmations and valence monitor options should be used for exact crystallographic disorder and untaken valence atoms. CHARMM (Chemistry at Harvard Macromolecular

Mechanics)It was used to minimize protein energy. The prepared protein was validated in the Ramachandran plots.



Figures.1. Schematic Representation of Ramachandran plots for Vitamin D receptors with PUFAs in EPA, DHA and AA.(a) 1RKG; (b) 1RKH; (c) 3VT7; (d)3M7R; (e)3VT3.

4.4. Identification of Active Site:

The receptor cavity method was used to predict the receptor protein's binding or active site, and even the inhibitory properties of the amino acid residues present in the binding sites (Andersson *et al.*, 2010).

4.5. Ligand Preparation:

Structure on n-3 PUFAs in VDR compounds Initial step for molecular docking studies. The omitted map of the advanced atomic model VDR, LBD H305Q is being used for fine ligand and electron significantly contributed to exposure. Calcitriol conformation is consistent to that observed in the crystal structure within the wild-type VDR in similar configurations (A-, Seco-B-, C- and D-rings). Subsequently, crystal structures of Ligand Binding Domain to the human VDR are identified during complex through VD and some fatty acids ligands exposed the C2 ligand is surrounded by H2O molecules that create a channel, which might also role in the stability of proteins. (N. Rochel et al.,2000). 15 In addition, manage the preserved water towards its mutant complex. Chemical structures of selected PUFAs are developed, modified, established and optimized with ChemSketch software and transformed into a 3D structure. Subsequently, using the Ligprep module, the ligand structure will be arranged in favor of docking. Throughout the whole of the tautomer as well as the isomers of the ligand, regard the isomer otherwise the lowest energy is found in the ligands' tautomers to be accepted as a docking design.

The Glide Standard Precision (SP) Ligand Docking

Standard Precision (SP) flexible ligand docking was accepted in Glide of (Schrödinger-Maestro v 10.1),(Venkatachalam C.M et al., 2003).Contained by which penalty be functional toward non-cis/trans amide bonds. Van der Waals scaling factor and partial charge cutoff be preferred to be (0.80 and 0.15), correspondingly used for ligand atoms. Final scoring is performed taken place energy-minimized poses and display as Glide score. Most excellent docked pose through lowest Glide score value be recorded designed for each ligand.

Table 1: Docking results compounds PUFAs on Vitamin D receptor (PDB id: 1RKG, 3M7R, 3VT3, 3VT7).

VDR Compound Names	Compound Id	Docking Score	Glide-Score
PDB ID: 1RKG	**DHA**	-10.216	-10.216
	AA	-10.571	-10.571
	EPA	-10.611	-10.611
PDB ID: 3M7R	**DHA**	-12.09	-12.09
	AA	-11.59	-11.59
	EPA	-11.449	-11.449
PDB ID: 3VT3	**DHA**	-10.66	-10.66
	AA	-10.574	-10.574
	EPA	-10.361	-10.361
PDB ID: 3VT7	**DHA**	-10.66	-10.66
	AA	-7.69	-7.69
	EPA	-7.632	-7.632

4.5. *In-silico* ADME Prediction via Lipinski rule of five:

The molecular properties of the drug that are important to its pharmacokinetics are how the drug is Absorption, distribution, metabolism, and excretion. (ADME). As a result, the sequentially, the lead structure is made more efficient for its dynamics and kinetics. Alterations in the molecular composition regularly lead to the compounds through high molecular weight, additional rings, bonding and efficient lipophilicity. The regulation states that in order to complete absorption otherwise access will become more likely while the ligand molecule violates the Lipinski rule of 5, so that it has added >5 H to the bond donor, the molecular weight is >500, the *log* P is >5, and addition of N, O is >10.(Lipinski CA et al., 2001 & Luscombe NM *et al.*, 2011). The similarity of the drug may be distinguished from the

complex stability of the distinct morphological properties, structure features, Evaluate a exact molecule were related to the identified compounds. They contain protein content, transport properties, protein similarity, reactivity, toxicity, metabolic stability as well as several others, mainly hydrophobic properties, distribution of electrons, hydrogen bonding properties, molecular size, and the molecule's occurrence distinct pharmacophoric characteristics influencing the performance on molecule during living organism. Demonstrate methodology for the study of drug similarity to potential ligands when It has an effect on the activities on the molecules in a living being, as well as bioavailability, transport characteristics, protein affinity, reactivity, toxicity, metabolic stability also included additional. They exhibited at the preferred PUFAs compounds under Lipinski's rule of 5 with Mol's inspiration.(http://www.molinspiration.com/).

TABLE.1: PREDICTION OF MOLECULAR PROPERTIES VIA LIPINSKI'S RULE

Compounds	No. of Hydrogen Acceptors	No. of Hydrogen Donors	A *Log* P	M.Wt.
EPA	02	01	5.6	302.5g/mol
DHA	02	01	6.2	328.5g/mol
AA	02	01	6.3	304.5g/mol

5. Molecular Dynamics Stimulation of Receptor- Ligand Complex:

The ligand- target complex's stability was investigated using a molecular dynamic simulation (Jo *et al.*, 2017). The simulation is set to NVT ensemble with a temperature range of 300K, Run: 100, Time step: 0.002, and QT (the temperature response set in a Molecular Dynamics simulation which enforces constant temperature) for 0.2. The simulation took 12.55 hours to complete.

6. Protein Preparation:

The crystal structure's 3D coordinates Vitamin D receptor (PDBID: 1RKG, 3M7R, 3VT3, 3VT7) be downloaded as of the RCSB PDB (http://www.rcsb.org/pdb). A global repository of information with reference to the proteins and nucleic acids in 3D. H_2O molecules follow, be distant on VDR PDB identified. The protein structure to be corrected through the use of conformation that alternates. To significant protein database has been subject to energy minimization through the strength field. After the energy minimization, the protein database will be subject to the depiction, in addition to the modification of binding site alternatives existing, the tool panel will take place on the way to the search besides possible binding site inside the VDR PDB Id's by strength field OPLS_2005, minimization were change the setting to the most extreme atom to 0.30Å on Root-Mean-Square-Deviation (RMSD).

7. Vitamin D receptor:

VDRs Crystal structures (PDB ID**: 1RKG, 3M7R, 3VT3, 3VT7, 3VT9**) download as of Root-Mean-Square-Deviation (RCSB) Protein Data Bank and prepare by eliminate to the nonessential water molecules, heteroatom there, small ions in addition to conformation that alternates. While the structure is finished, modeling into missing loops. A standardized description of the atom has also been inserted into the missing atom and titrable residues were protonized by the pKa prediction. In terms of its RMS gradients, potential energy, Vander Waals' energy, electrostatic energy, and in complexes were being checked prior to protein minimization.

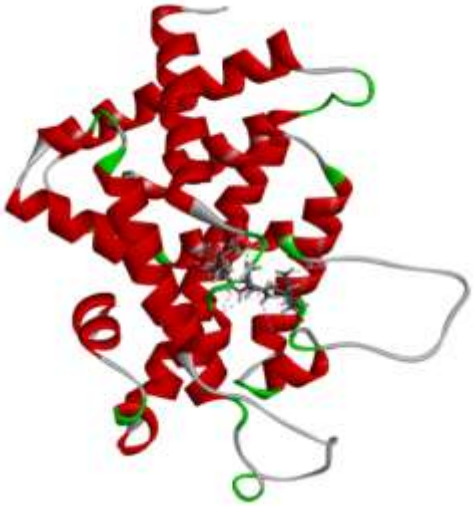


Figure.2. Represent the Crystalline Structure on VDR.

9*. In-silico* Molecular docking studies:

In-silico docking studies have carried out with the Schrodinger Software Protocol. Interaction with particular PUFAs compounds through a protein is evaluated somewhere the ligands remain flexible in addition to protein. To minimize the structures on selected PUFAs, its input ligand was used. Dock score and Glide score schemes for dock ligands were applied to the receptor-binding site. Dock score and Glide score schemes would be applied for the docking of ligands at the receptor binding site as well. We're being created in the process. by random ligand conformations-high-temperature MD. Random rigid-body rotations and annealing of the binding site will transform the conformations into their compounded forms. Significantly less energy is required to process the ligand poses. For each position, we utilized DOCKER ENERGY (the sum of the internal ligand strain energy and the receptor-ligand non-bonded interaction energy) and DOCKER INTERACTION ENERGY (the non-bonded interactions between the ligand and receptor. In any event, the binding model with the lowest energy values was found to be the most promising.

10. Results & Discussions

10.1. Crystal Structures

Vitamin D receptors in the crystal structures in the LBD of PUFAs and VDR in the ternary complexes with peptide contain the target sequence of PUFAs in addition to the ligands

determined by the ligand individuals on LBD VDR in ternary complexes within the peptide contain the target sequence of nuclear receptor coactivators and mutations The structure reveals that Although 1, 25 $(OH)_2D_3$ and its metabolites bind to the same VDR LBP as the LCA, the direction of the binding is reversed.(Nandhikonda *et al.*, 2013).

10.2. Molecular Docking Analysis

Responsibility for computational analysis, glide docking, is used to investigate the binding mode of The α- amylase enzyme. Both the glides standard precision (SP), extra precision (XP) mode has recommend wherever XP mode is utilize to support cross-validation. (Veeramachaneni GK et al., 2015). Grid-based docking study should be used on the analyze how molecules are bound by amino acids found within the protein's active site. In order toward recognize lead compound for anti-diabetic treatment, were also subject to a docking studies of the active PUFAs taking place even at active VDR site. In order to study the interaction with its compound through the Vitamin D receptor Binding Pockets (PDB id: 1RKG, 3M7R, 3VT3, 3VT7). While executing Glide Docking Analysis through Schrodinger Suite v10.1, where, along with the Omega-3 PUFAs (EPA, DHA and AA) compounds, the maximum docking score against the associated enzymes is also shown. The docking score recommended in such a case that the PUFAs (EPA, DHA and AA) have the maximum affinity to the VDR equivalent in the other compounds. The docking study's findings are detailed in of the docking figures and are displayed as.

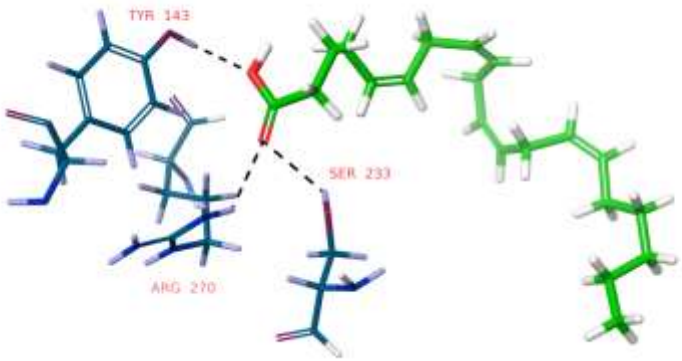


Figure. 3. PDB ID: 1RKG, Arachidonic Acid (AA) interaction with VDR Residues

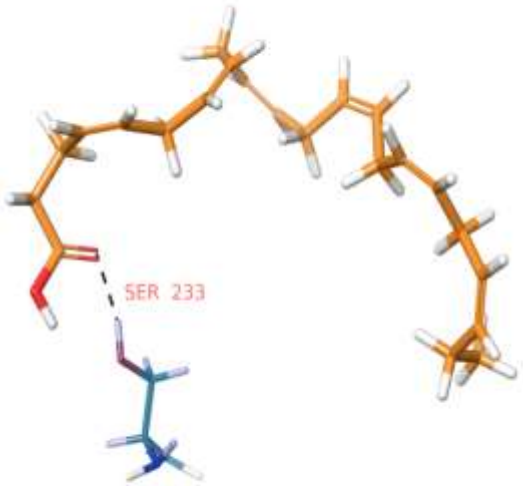


Figure.4. PDB ID: 1RKG, Docosahexaenoic Acid (DHA) interaction with VDR Residues

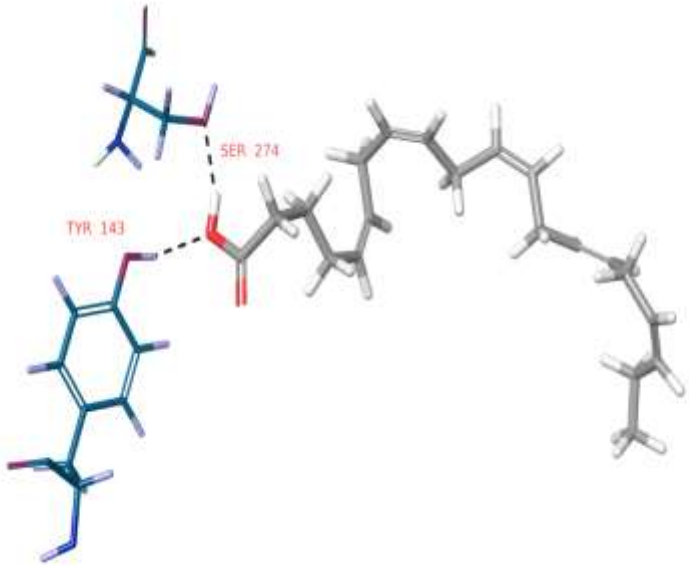


Figure.5. PDB ID: 1RKG, Eicosapentaenoic Acid (EPA) interaction with VDR Residues

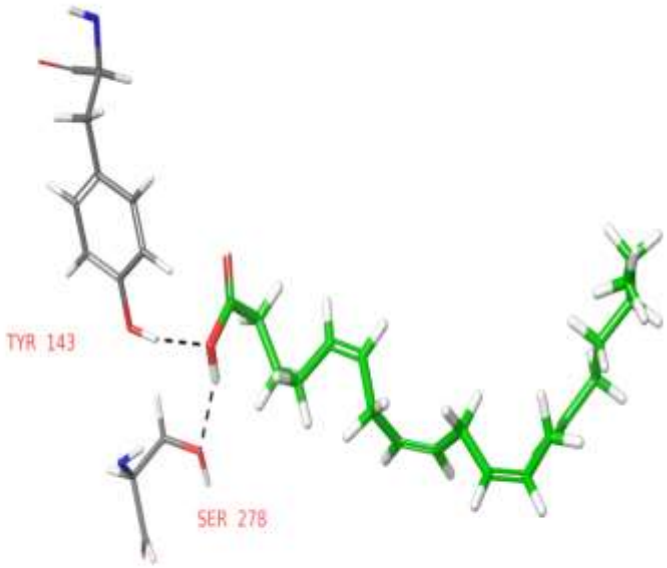


Figure. 6. PDB ID: 3M7R, Arachidonic Acid (AA) interaction with VDR Residues

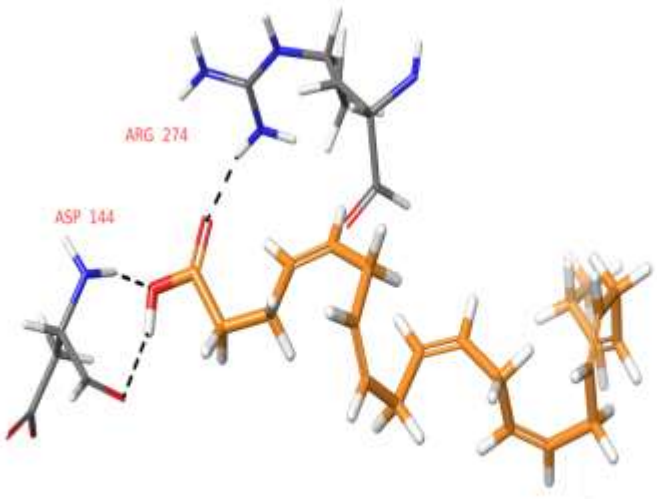


Figure.7. PDB ID: 3M7R, Docosahexaenoic Acid (DHA) interaction with VDR Residues

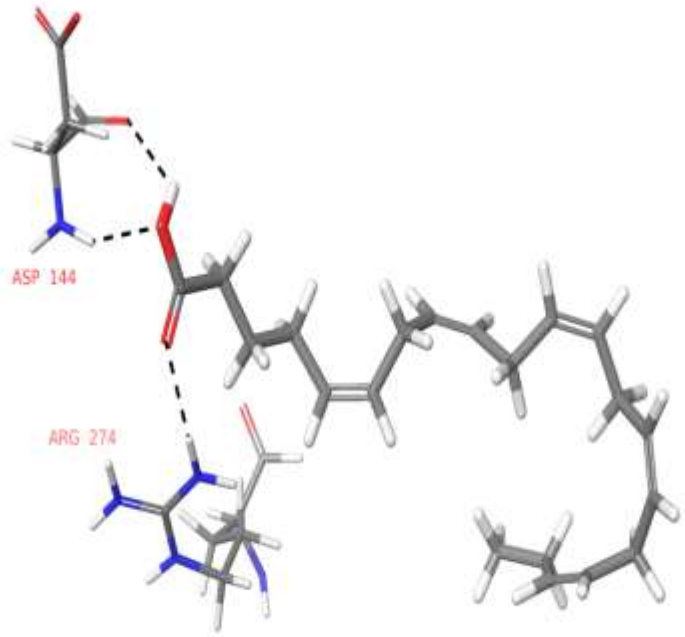


Figure. 8. PDB ID: 3M7R, Eicosapentaenoic Acid (EPA) interaction with VDR Residues

Figure. 9. PDB ID: 3VT3, Arachidonic Acid (AA) interaction with VDR Residues

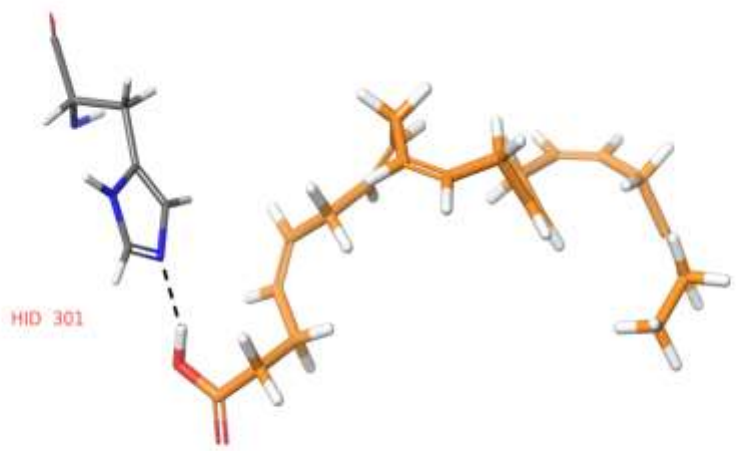


Figure.10.PDB ID: 3VT3, Docosahexaenoic Acid (DHA) interaction with VDR Residues

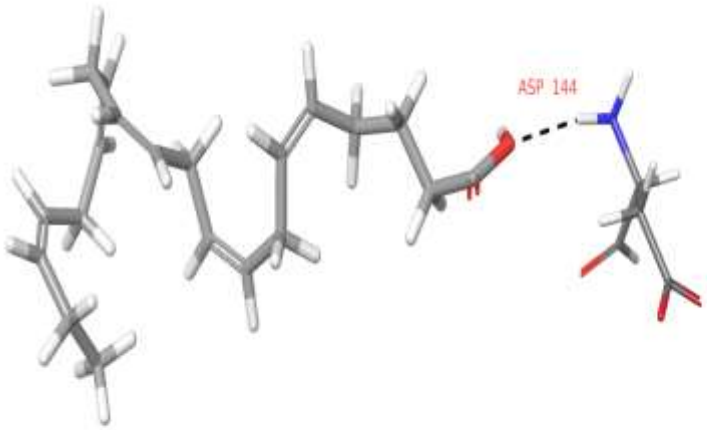


Figure. 11. PDB ID: 3VT3 Eicosapentaenoic Acid (EPA) interaction with VDR Residues

Figure. 12. PDB ID: 3VT7 Arachidonic Acid (AA) interaction with VDR Residues

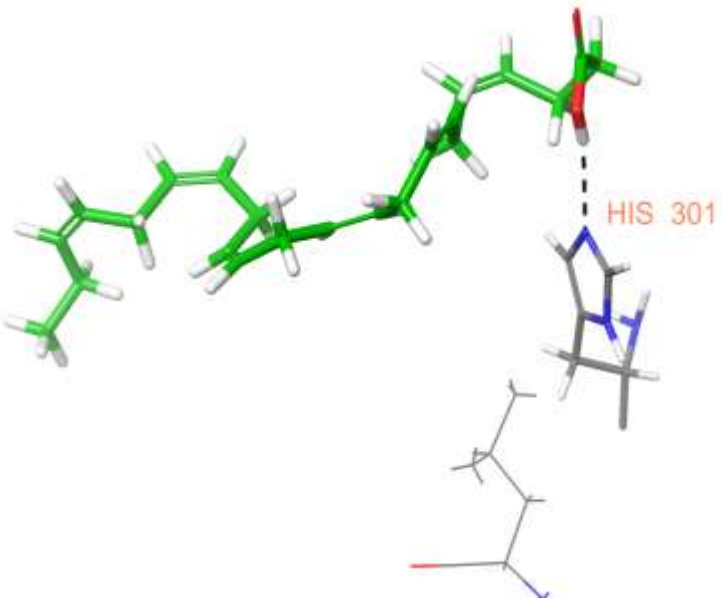


Figure.13. PDB ID: 3VT7, Docosahexaenoic Acid (DHA) interaction with VDR Residues

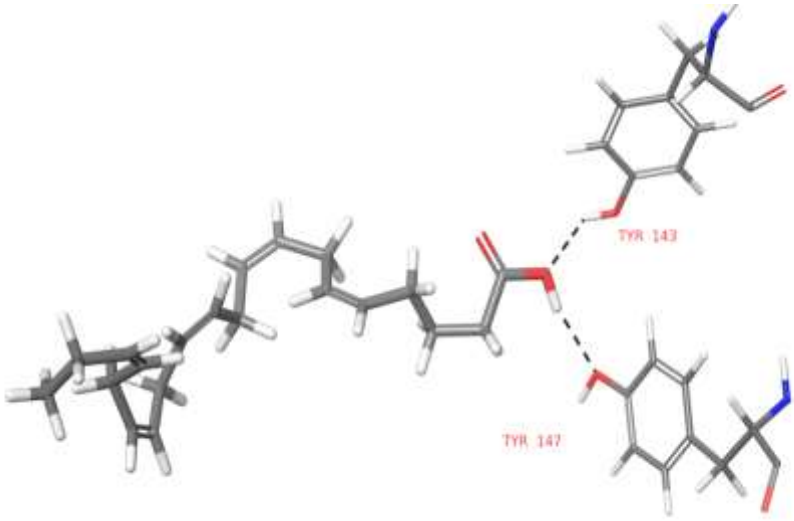


Figure.14. PDB ID: 3VT7, Eicosapentaenoic Acid (EPA) interaction with VDR Residues

Schematic Diagram illustration to the interactions between the best pose initiate to the selected compounds with vitamin D receptor (PDB ID: 1RKG, *1RKH, 3M7R, 3VT3, 3VT7*). **The PDB ID: 1RKG** colors indicate the residue (or species) type: Red-acidic (Ser274a, Ser233a), Green-hydrophobic (Leu226, Val296, Leu229, His301), Purple-basic (Tyr143), Blue-polar (Ser233, Arg270), Light gray-other (Gly, water), and Darker gray-metal atoms. Figure. 15. 1RKG.

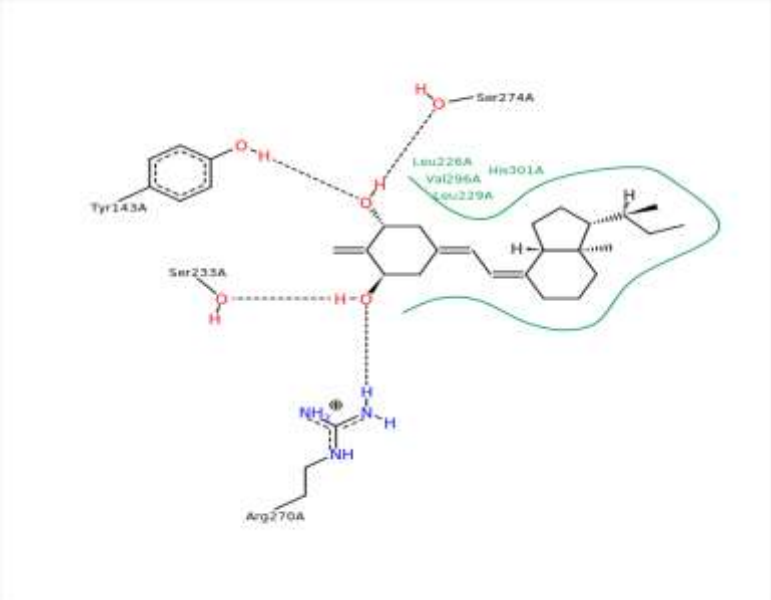


Figure.15. PDB ID: 1RKG

PDB ID. 1RKH: Colors indicate the residue (or species) type: Red-acidic (Ser274a, His393a, Ser233a), Green-hydrophobic (Val296a), Green-hydrophilic (Leu309a, His301a, Ile267a, Val230a, Leu229a) Purple-basic (Tyr143a, Arg270a, His301a, His393a), Blue-polar (Arg270a), Figure.16.

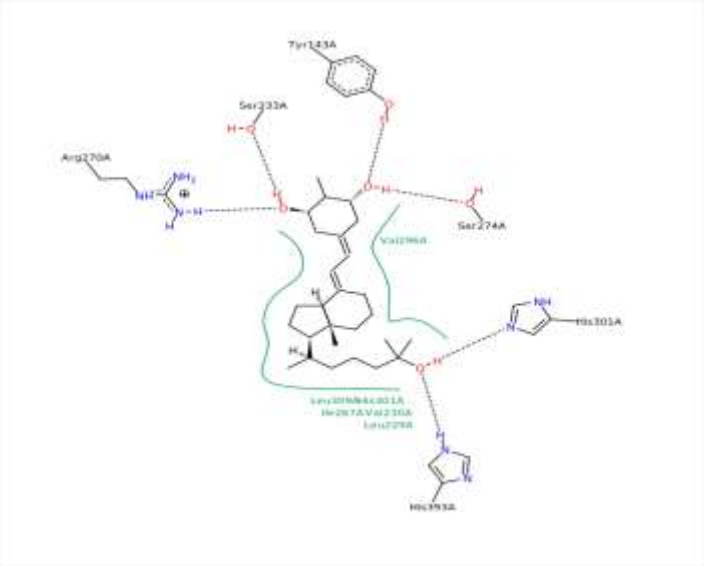


Figure.16. PDB ID: 1RKH

Arachidonic Acid (AA): Colors indicate the residue (or species) type: Red-acidic (Arg270), Green-hydrophobic (Tyr397, Leu410, Leu400, Leu223, Leu226, Ala227, Leu229, Val230, Tyr232, Leu309, Leu305, Ile264, Ile264. Ile267, Met268, Tyr143),Green-hydrophilic (Ala299, Phe418, Tyr291, Val296), Blue-polar (Hid301, Hid393),Dark blue color(Ser271, Ser274) Light gray-other (Gly, water), and Darker gray-metal atoms. Figure17.

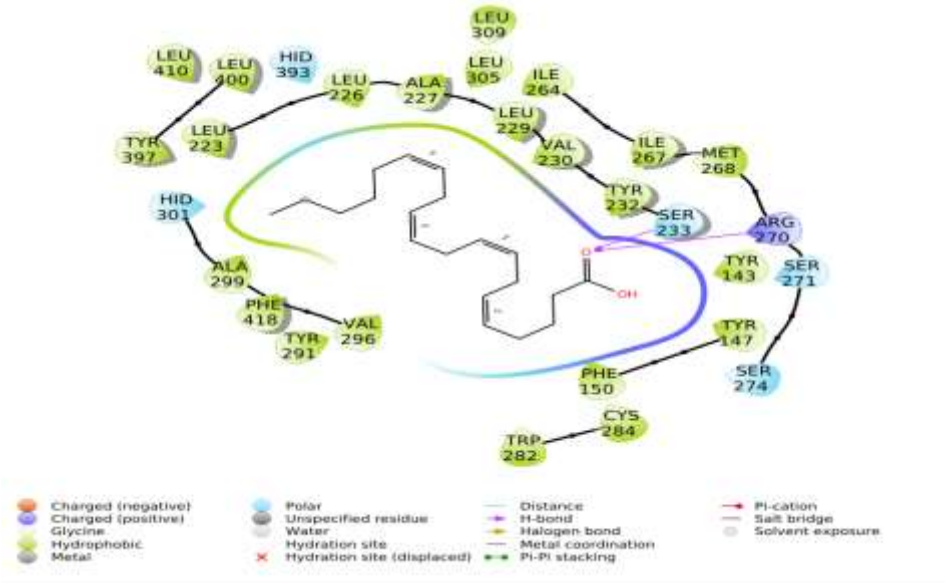


Figure.17.PDB ID: 1RKH- Arachidonic Acid (AA) interaction with VDR Residues

Docosahexaenoic Acid(DHA): Colors indicate the residue (or species) type: Green-hydrophobic (Tyr397, Leu410, Leu400, Leu223, Leu226, Ala227, Leu229, Val414, Phe418, Leu410, Leu223, Leu226, Ala227, Leu229, Val230, Val296),Green-Hydrophilic (Leu305tyr291, Ile264, Ile267, Met268, Leu309, Trp282, Cys284), Blue-Polar (HID301, HID393, SER233),Dark blue color(Arg270) Light gray-other (Gly, water),and Darker gray-metal atoms. Figure.18.

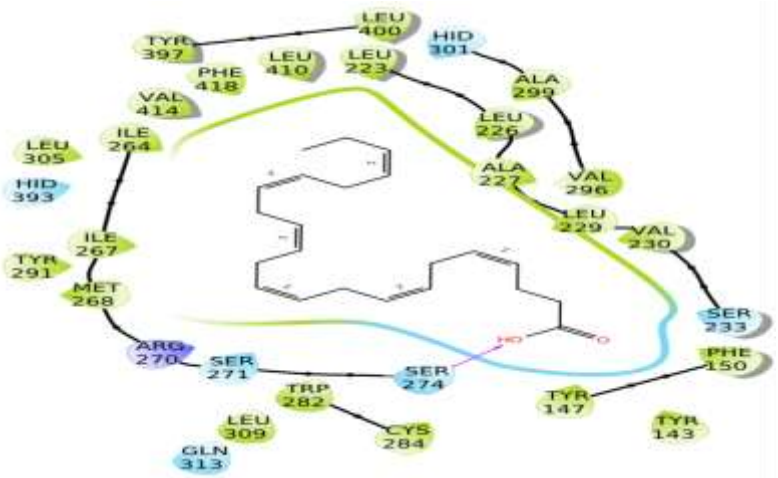


Figure.18. PDB ID: 1RKH- Docosahexaenoic Acid (DHA) interaction with VDR Residues

Eicosapentaenoic Acid(EPA): colors indicate the residue (or species) type: Red-acidic(Ser233, Arg270), Green-hydrophobic (Trp282, Cys284, Tyr143, Met268, Ile267, Phe150, Tyr147),Green-hydrophilic (Tyr291, Val230, Leu229, Leu226, Val296, Ala299, Leu305, Ile306, Leu309), Blue-Polar (Hid301, Hid393, Ser233, Ser274, Ser271),Dark blue color(Arg270) Light gray-other (Gly, water), and Darker gray-metal atoms, Figure. 19.1RKH.

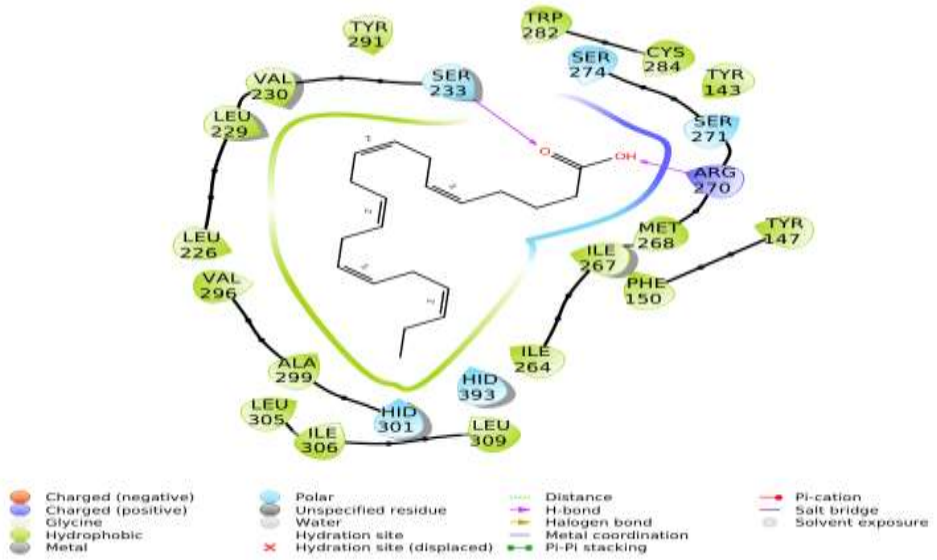


Figure.19. PDB ID: 1RKH- Eicosapentaenoic Acid (EPA) interaction with VDR Residues

PDB ID: 3M7R: colors indicate the residue (or species) type: Red-acidic (Asp144), Green-hydrophobic (Cys288a, Trp286a, Tyr236, Val234, Leu233, Leu 230, Val300, Tyr147, Phe150, Leu309),Green-hydrophilic (Leu313a, Tyr295a, Ile271a, Met272a), Blue-polar (Ser237a, Ser275a, Ser278a, Thr142a, Gln317a, Gln305a),Dark blue color (Lys240A, Arg274A) Light gray-other (Gly, water), and Darker gray-metal atoms, Figure.20.

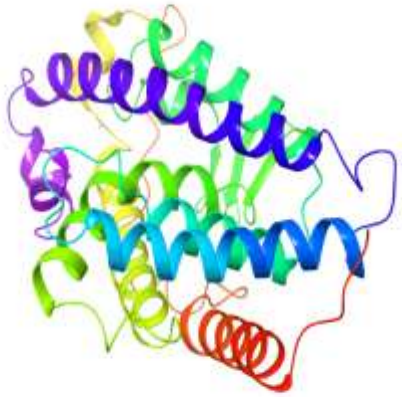


Figure.20, PDB ID: 3M7R

Arachidonic Acid(AA): colors indicate the residue (or species) type: Red-acidic (SER278), Green-hydrophobic (Try147, Tyr143, Phe150, Tyr295, Cys288, Trp286, Met272, Ile271, Ile268, Leu309),Green-hydrophilic (Val234, Leu233, Ala231, Leu230, Leu414,Tyr401, Leu404, Phe422), Blue-polar (Hid397, Gln305, Ser275, Ser278),Dark blue color (Arg274) Light gray-other (Gly, water), and Darker gray-metal atoms**,** Figure.21.

Figure.21. PDB ID: 3M7R- Arachidonic Acid (AA) interaction with VDR Residues

Docosahexaenoic Acid(DHA): Colors indicate the residue (or species) type: Red-acidic (HID301), Green-hydrophobic (Tyr143, Val230, Leu229, Ala227, Leu226, Leu223, Leu410, Val414, Phe418, Tyr397, Leu400, Ile264, Ile267, Met268),Green-hydrophilic (Tyr143, Phe150, Cys284, Try282, Tyr291), Blue-polar (Ser233, Ser274, Ser271, Gln313),Dark blue color (HID393,HID301) Light gray-other (Gly, water), and Darker gray-metal atoms. Figure.22.

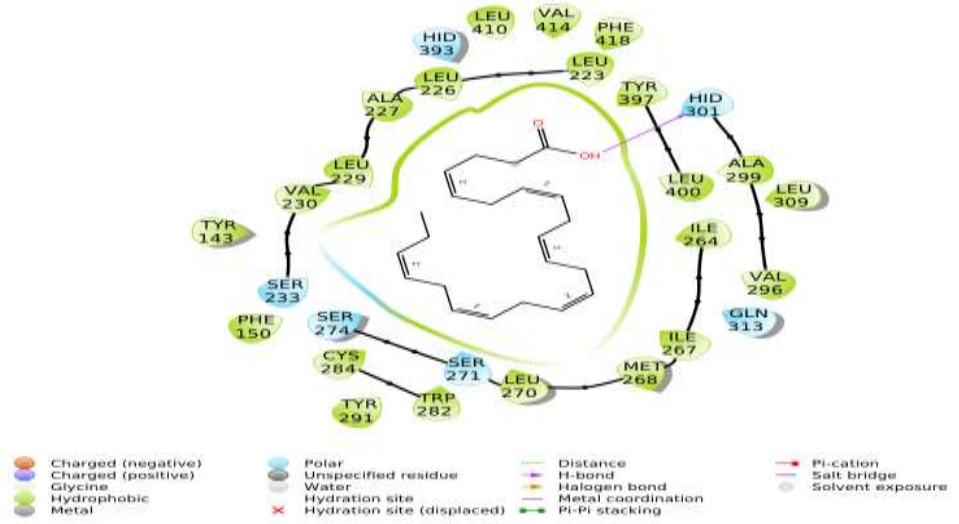


Figure.22. PDB ID: 3VT3 – Docosahexaenoic Acid (DHA) interaction with VDR Residues

Eicosapentaenoic Acid(EPA): Colors indicate the residue (or species) type: Red-acidic (ASP144), Green-hydrophobic (Cys288, Trp286, Tyr236, Val234, Leu233, Leu230, Val300, Tyr143, Tyr147, Phe150),Green-hydrophilic (Met272, Ile271, Ile268, Tyr295), Blue-polar (Ser275, Ser237, Ser278, Thr142, Gln305, Hid397, Gln317),Dark blue color (LYS240, ARG274) Light gray-other (Gly, water), and Darker gray-metal atoms, Figure. 23.

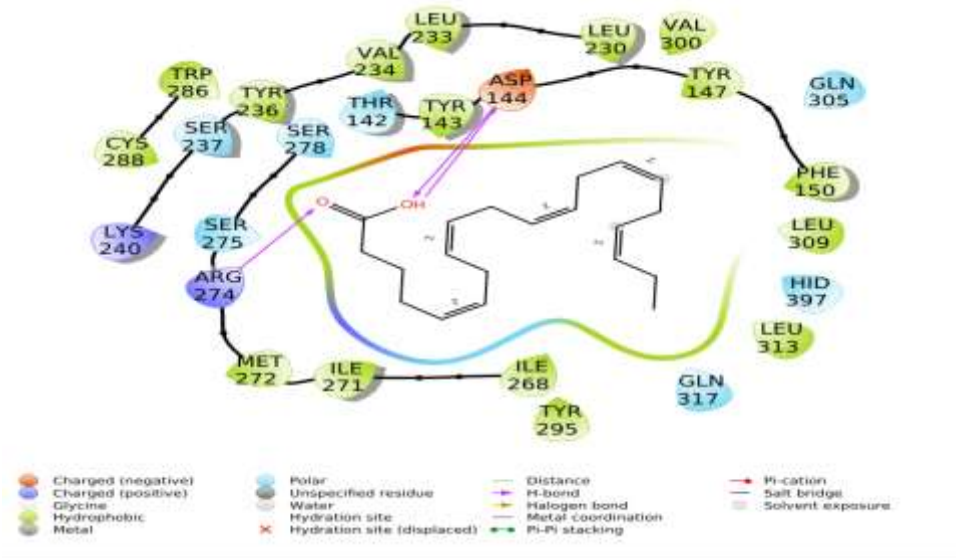


Figure.23. PDB ID: 3M7R- Eicosapentaenoic Acid (EPA) interaction with VDR Residues

PDB ID :3VT3 Arachidonic Acid(AA): colors indicate the residue (or species) type: Red-acidic (Ser278), Green-hydrophobic (Tyr147, Tyr143, Phe150, Tyr295, Cys288, Trp286met272, Ile271, Ile268, Leu313, Leu309),Green-hydrophilic (Val234, Leu233, Ala231, Leu230, Leu227, Val418, Tyr401, Leu404, Leu414), BLUE-POLAR (Ser237, Gln 305, Hid397, Se278, Ser275),Dark blue color (ARG274) Light gray-other (Gly, water), and Darker gray-metal atoms. Figure. 24.

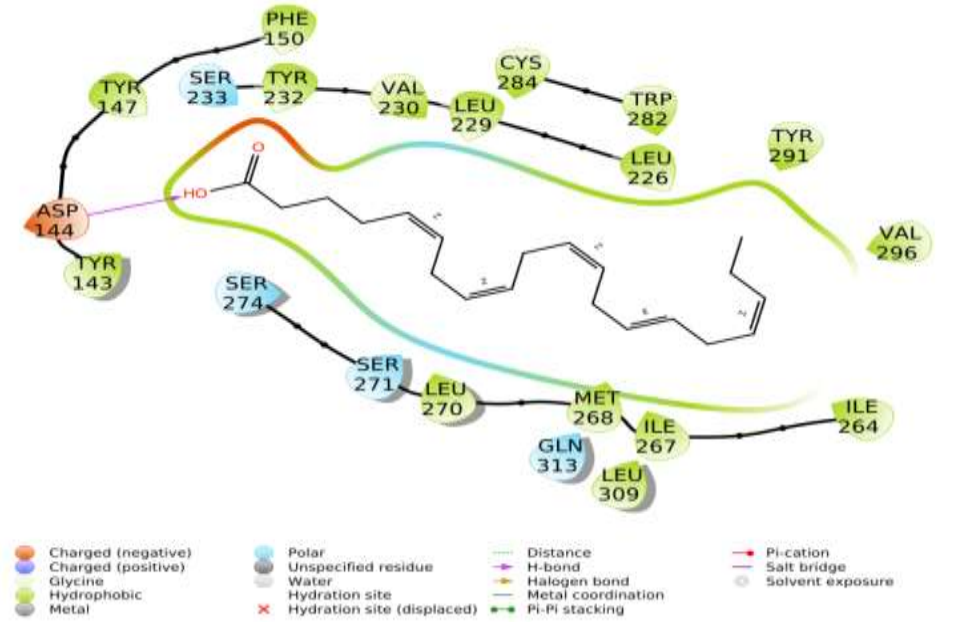


Figure.24.PDB ID: 3VT3 – Arachidonic Acid (AA)interaction with VDR Residues

Docosahexaenoic Acid (DHA): colors indicate the residue (or species) type: Red-acidic (HID301), Green-hydrophobic (Thr143, Val230, Leu229, Ala227, Leu226, Leu 410, Val414, Phe418, Leu223, Tyr397, Leu400, Ile264, Ile267, Ala299, Val296, Leu309),Green-hydrophilic (Thr143, Phe150, Cys284, Tyr291, Trp282, Leu270, Met268, Ile267, Ile264), BLUE-POLAR (Ser233, Ser274, Ser271, Hid301, Gln313),Light gray-other (Gly, water), and Darker gray-metal atoms. Figure.25.

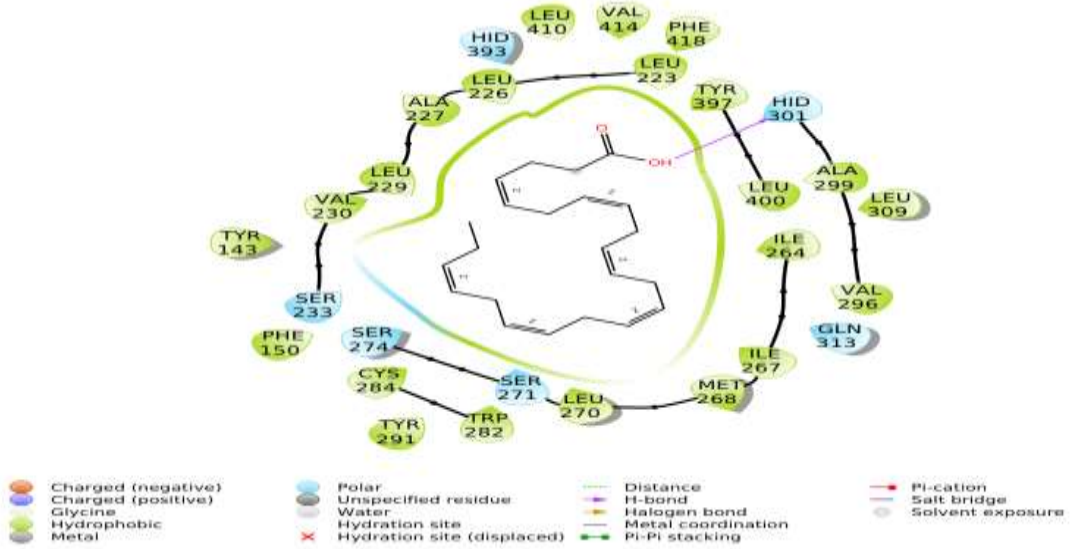


Figure.25. PDB ID: 3VT3 – Docosahexaenoic Acid (DHA) interaction with VDR Residues.

Eicosapentaenoic Acid (EPA): colors indicate the residue (or species) type: Green-hydrophobic (Val414, Phe418, Leu305, Ala299, Ile264, Ile267, Met268, Tyr291, Phe150, Tyr147, Tyr143, Tyr397, Leu400),Green-hydrophilic (Leu223, Leu410, Leu226, Ala227, Leu229, Val230, Leu309), Blue-polar (His301, Ser271, Ser274, His393, Ser233),Dark blue color(ARG270)Light gray-other (Gly, water), and Darker gray-metal atoms. Figure.26.

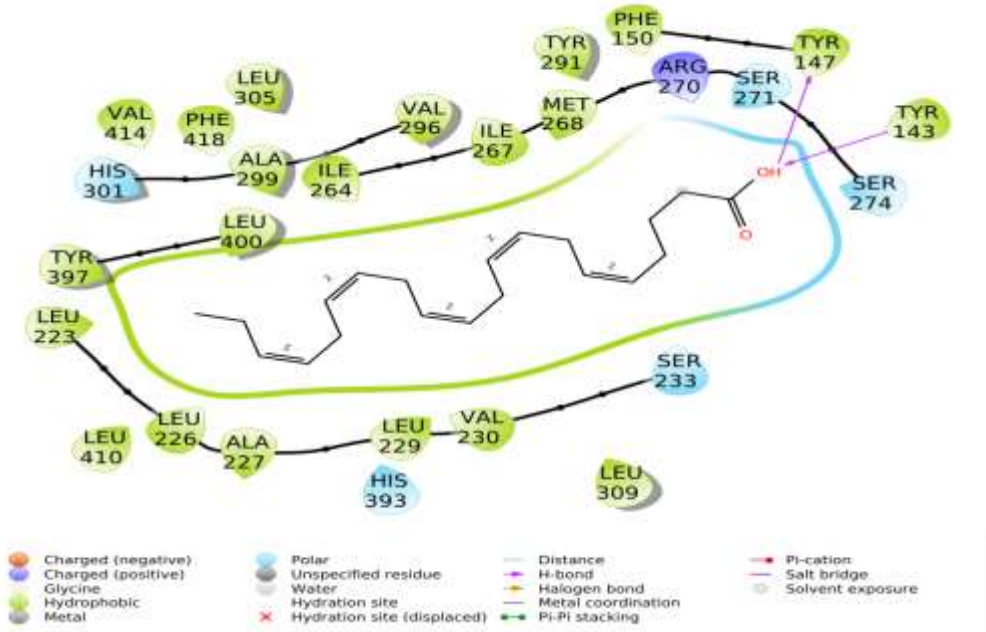


Figure.26. PDB ID: 3VT3 - Eicosapentaenoic Acid (EPA) interaction with VDR Residues.

PDB ID: 3VT7: Colors indicate the residue (or species) type: Red-acidic (Ser233), Green-Hydrophobic (Leu223, Leu226, Ala227, Leu229, Val230, Tyr232, Leu410, Leu400, Tyr 397, Leu309, Tyr 143, Tyr147, Phe150),Green-hydrophilic (Val 414a, Phe418a, Ala299, Val296, Ile264, Tyr291, Ile267, Met268), Blue-polar (His301, His393),Dark blue color (ARG270A) Light gray-other (Gly, water), and Darker gray-metal atoms**.** Figure.27.

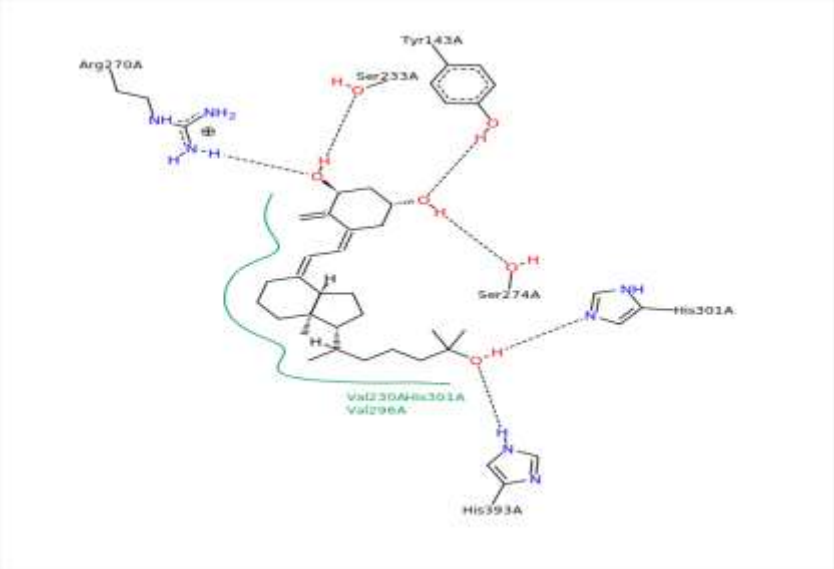


Figure.27. PDB ID: 3VT7

Arachidonic Acid (AA): colors indicate the residue (or species) type: Red-acidic (Tyr143), Green-hydrophobic (Tyr397, Leu400, Phe150, Tyr147, Leu410, Val414 Ile267),Green-hydrophilic (Phe418, Leu223leu226, Ala227, Leu229, Val230,Leu 309), Blue-polar (His301, His393 Ser233, Ser274, Ser271), Dark blue color (Arg270a) Light gray-other (Gly, water), and Darker gray-metal atoms**.** Figure.28.

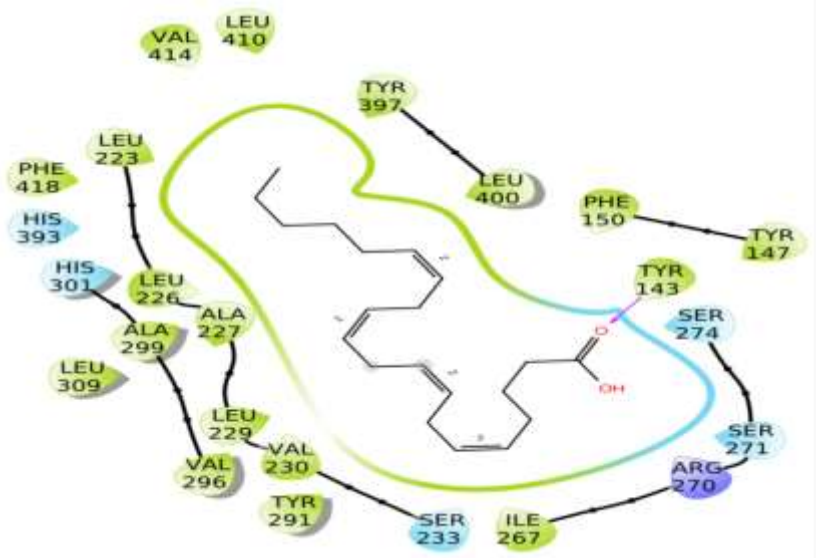


Figure.28. PDB ID: 3VT7- Arachidonic Acid (AA) interaction with VDR Residues.

Docosahexaenoic Acid (DHA): colors indicate the residue (or species) type: Green-hydrophobic (Leu410, Phe418, Tyr397, Leu400, Val230, Leu229, Ala227, Leu226, Leu223, Val296, Ala299, Val 414),Green-hydrophilic (Tyr291, Met268, Ile267, Ile264, Leu309, Leu305), BLUE-POLAR (His301, His393 Ser233, Ser274, Ser271), Dark blue color (SER271) Light gray-other (Gly, water), and Darker gray-metal atoms. Figure.29.

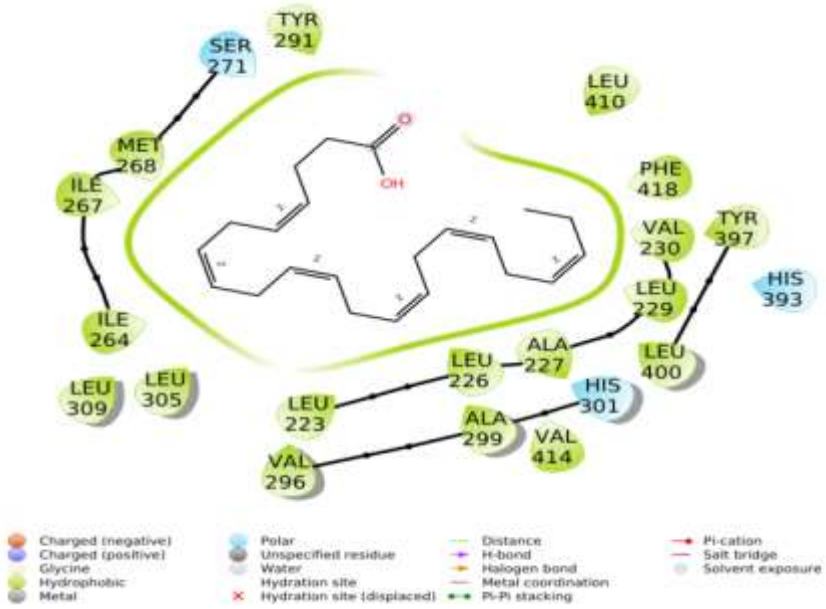


Figure.29. PDB ID: 3VT7- Docosahexaenoic Acid (DHA) interaction with VDR Residues.

Eicosapentaenoic Acid (EPA): colors indicate the residue (or species) type: Red-acidic (Tyr147, Tyr143), Green-hydrophobic (Val414, Phe418, Leu305, Ala299, Val296, Ile264, Ile267, Met268, Tyr291, Phe150, Tyr397, Leu400),Green-hydrophilic (Leu223, Leu226, Ala227, Leu229, Val230, Leu309, Leu410), Blue-polar (His301, His393 Ser233, Ser274, Ser271),Dark blue color (Arg270) Darker gray-metal atoms, and light gray-other (Gly, water). Lines between ligand atoms and protein residues indicate interactions with the protein: Green—pi-pi stacking interactions, Orange-pi-Cation interactions, Solid pink—H-bonds to the protein backbone, Dotted pink—H-bonds to protein side chains, Green—pi-pi stacking interactions Gray spheres indicate ligand atoms that are exposed to solvent. A line surrounding the ligand is colored with the color of the adjacent protein residue to represent the protein "pocket." The pocket's entrance is shown by the gap in the line.(**Fig. 30. 3VT7**)

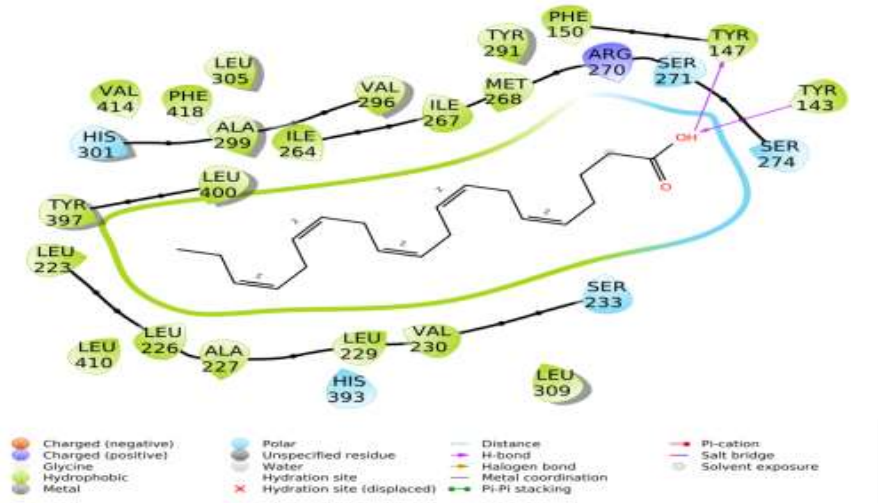


Figure.30. PDB ID: 3VT7- Eicosapentaenoic Acid (EPA) Interaction with VDR Residues.

Conclusion

Select Long Chain Omega- 3 and Omega- 6 PUFAs control VDR at LBD with distinct binding energies and interactions, as shown by our *In-silico* molecular docking studies. Furthermore, they worked in mutants of the Vitamin D Receptor. PUFAs and VDR are therefore proposed as possible modulators of T2DM on the basis of this research.

Implication of the Proposed Hypothesis:

The proposed hypothesis and accompanying in-silico evidences give a strong foundation for the mechanism by which selective PUFAs regulate VDR; this would be the definitive pioneering investigation of the complementary functions of selective PUFAs (EPA, DHA, and AA) in metabolic targets. The findings will be used to create a new treatment/prevention method for Type 2 diabetes, which would have a substantial health effect in treating VDD and its related consequences.

Abbreviations:

VD- Vitamin D; VDR- Vitamin D receptor; EPA- Eicosapentaenoic acid; DHA- Docosahexaenoic Acid; AA- Arachidonic Acid; PUFAs- Polyunsaturated fatty acids; ADMET-

Absorption, Distribution, Metabolism and Excretion Test; RCSB - Research Collaboratory for Structural Bioinformatics; PDB - Protein Data Bank; T2DM- type 2 Diabetes mellitus.

Conflict of Interest

Authors declare no conflict of interest

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